REMARKS

The Examiner is thanked for the due consideration given the application. This amendment is being filed concurrent with a request for continued examination.

Claim 36 is pending in the application. Claims 1-35 and 37-48 have been canceled.

Amended claim 36 now recites a process for preparing a monoclonal antibody directed against endothelial cells with an angiogenic phenotype. It has been added that the endothelial cells used in the claimed process are derived from an aorta, and the cells are cultured in a medium supplemented with oestradiol and VEGF. Also, the "consisting essentially of" language restricts the active components to the supplement to oestradiol and VEGF.

Amended claim 36 finds support in the specification at page 7, lines 19-21 and at page 18, lines 12-13.

No new matter is believed to be added to the application by this amendment.

Rejection Over MAYUMI et al.

Claim 36 has been rejected under 35 USC §102(b) as being anticipated by MAYUMI et al. (U.S. Patent 6,440,733). This rejection is respectfully traversed.

MAYUMI et al. pertain to monoclonal antibody recognizing antigens expressed on the surface of ***tumor vessel endothelial cells***.

MAYUMI et al. also pertain to a process for producing the monoclonal antibody. In particular, it is mentioned in MAYUMI et al. that the process includes the steps of:

- immunization of animal with tumor endothelial cells, preferably with membrane fraction of the tumor endothelial cells,

- preparation of hybridomas, and

- checking inhibiting properties of obtained antibodies.

MAYUMI et al. is illustrated by the production of anti CD44 monoclonal antibodies, named TES antibodies, that inhibit cell proliferation of KTM-17 transplanted cells, i.e., by inhibiting the neovascularization of the ***KTM-17 derived tumor.***

In contrast, instant claim 36 of the present invention sets forth endothelial cells that have an angiogenic phenotype and are derived from an aorta. This definition implicitly indicates that endothelial cells used to obtain endothelial cells with an angiogenic phenotype are “normal” endothelial cells but not tumoral endothelial cells.

The cells derived from aorta are cultured in a medium supplemented with a hormone (oestradiol) and only one growth factor: Vascular Endothelial Growth Factor (VEGF). From this culture in such a medium, endothelial cells derived from aorta acquire an “activated phenotype”, and this activated phenotype being strictly induced by the presence of the only VEGF.

On the other hand, MAYUMI et al. disclose the use of endothelial cells derived from animals (WKAH/hkm rats) subcutaneously injected with KTM-17 fibrosarcoma.

More precisely, the endothelial cells used in MAYUMI et al. are taken off from the neo vasculature developed in the grafted KMT-17-induced tumor.

In contrast to the cells used in the present invention, MAYUMI et al. cells have been "activated" by KMT-17 cells, which excrete ***some*** factors, whereas the present invention's endothelial cells from an aorta are cultured ***only*** with VEGF + oestradiol.

Therefore, the cells of the present invention, regarding their angiogenic phenotype, differ from those of MAYUMI et al. since they are "activated" with different growth factors.

Also, MAYUMI et al. disclose the use of "Normal" vessel endothelial cells from rat fat tissue for preparing non-relevant serum, in order to mask epitopes that are present both in normal endothelial cells and tumoral endothelial cells.

These normal endothelial cells are cultured without adding VEGF, as discussed in column 9, second paragraph of Example 1.

Thus, these cells are also different from the endothelial cells used in the present invention, since they are un-activated (without angiogenic phenotype) and cultured with neither VEGF nor oestradiol.

MAYUMI et al. thus fail to teach each and every element of claim 36 of the present invention. MAYUMI et al. therefore fail to anticipate claim 36 of the present invention.

This invention is believed to be overcome, and withdrawal thereof is respectfully requested.

Rejection Over BURROWS et al.

Claim 36 has been rejected under 35 USC §102(b) as being anticipated by BURROWS et al. (*Clin. Cancer Research*, 1995 Dec; 1912): 1623-34) as evidenced by PELLIZZARO et al. (*Carcinogenesis* 23(5); 735-740, 2002). This rejection is respectfully traversed.

BURROWS et al. pertain to a monoclonal antibody directed against Endoglin, a protein present at the surface of endothelial cells.

BURROWS et al. describe a process of producing this monoclonal antibody. The process described in BURROWS et al. includes a step of immunizing mice with HUVEC endothelial cells, the HUVEC endothelial cells being previously stimulated with a HT-29 cells-conditioned medium. HT-29 cells are known to secrete angiogenic factor angiogenin (see reference FETT el al. *Biochemistry* 24: 5480-5486, 1985, cited in BURROWS et al.).

BURROWS et al. also refer to PELLIZZARO et al., which disclose that HT29 cells secrete VEGF as the main pro-angiogenic factor.

Thus, from the teaching of BURROWS et al. and PELLIZZARO et al. one would be led to believe that conditioned medium derived from HT-29 cells contains at least 2 proangiogenic factors including VEGF and angiogenin.

However, in instant claim 36 of the present invention, cells used to obtain endothelial cells with an angiogenic phenotype are dependant upon ***only*** VEGF as pro-angiogenic factor, and oestradiol.

Thus, since the instant claim 36 of the present invention sets forth only the use of angiogenic cells cultured only in the presence of VEGF and oestradiol, the use of the growth factors being absent from the teaching of BURROWS et al. and/or PELLIZZARO et al., claim 36 is novel over the applied art.

BURROWS et al. (as evidenced by PELLIZZARO et al.) thus fail to teach each and every element of claim 36 of the present invention. BURROWS et al. therefore fail to anticipate claim 36 of the present invention.

This invention is believed to be overcome, and withdrawal thereof is respectfully requested.

Rejection Under 35 USC §103(a)

Claim 36 has been rejected under 35 USC §103(a) as being unpatentable over MAYUMI et al. in view of HEBBEL et al. (U.S. Publication 2002/0042130). This rejection is respectfully traversed.

The Official Action asserts that HEBBEL et al. teaches that endothelial cells are cultured in a medium containing angiogenic factors such as ECGF or VEGF only.

As has been noted, MAYUMI et al. disclose a process for preparing antibodies that recognize an element expressed at the cell surface of endothelial cells with an angiogenic phenotype, the cells being derived from a tumor (tumoral endothelial cells with an angiogenic phenotype).

From the combination of the teaching of MAYUMI et al. and HEBBEL et al., a skill artisan would be taught to use tumoral endothelial cells derived from KMT-17 tumor neo vessels, and would culture theses cells in a medium containing angiogenic agent, such as VEGF.

However, the present invention sets forth a process for producing antibodies that inhibit angiogenesis, the antibodies being obtained by immunizing animals with “normal” endothelial cells that have been expended and activated in vitro only with VEGF and oestradiol.

The present invention is based on the unexpected observation that “normal” endothelial cells derived from an aorta can be “activated” in *vitro* by using only one pro-angeogenic factor VEGF, in combination with oestradiol,

In contrast, endothelial cells with an angiogenic phenotype derived from a tumor correspond classically to “normal”

cells activated with ***many*** angiogenic factors, among which VEGF takes an important role.

In the present invention, the addition of pro-angiogenic factor VEGF is controlled (addition of a specific dose), whereas, in an animal developing a tumor, pro-angiogenic factors are secreted by said tumor at a dosage depending of each type of tumor.

In the present invention, an important fact is that "normal" cells are activated with a specific set of angiogenic factors, such that the resulting activated cells acquire a determined phenotype corresponding to a particular cellular response to the angiogenic factors.

For example, an endothelial cell activated by VEGF and FGF would express protein at the cell surface that result from the combination of the nuclear response of the two angiogenic factors. In contrast, the same cell stimulated by only one of these two angiogenic factors would share a cell surface proteins' pattern which is completely different from the pattern of surface protein in response to the other angiogenic factor, or a combination of both angiogenic factors.

The concept of specific cellular response to growth factor determined stimulation is well known in the art, and illustrated for example in Marks, F. et al. (2008). *Cellular Signal Processing – An Introduction to the Molecular Mechanisms of Signal Transduction, First Edition*, or in Alberts, B. et al.

(2007) *Molecular Biology of the Cell, Fifth Edition* (Garland Science; Taylor & Francis Ltd, 4 park Square, Milton Park, Oxfordsbire OXl4 4RN, UK.)

Thus, MAYUMI et al. teach the use of tumoral cells with an angiogenic phenotype. These cells express at the cell surface a combination of proteins that corresponds to the nucleus response to all the angiogenic factors received by the cell. In the restricted form of the teaching of US patent MAYUMI et al., tumoral endothelial cells express at the cell surface a combination of proteins corresponding to the gene expression in response to VEOF and angiogenin

From the combination of the teaching of MAYUMI et al. and of HEBBEL et al., one would believe that cells expressing a protein pattern corresponding to gene expression in response to VEGF and angiogenin are cultured in VEGF, and thus, are “re”-activated by culture in a medium containing VEGF. These resulting cells would have a protein pattern corresponding to gene expression in response to VEGF and angiogenin, in which protein expression profile corresponding to VEGF would be enhanced compared to the cells Un “re”-activated cells.

However, the claim 36 of the present invention only recites the use of endothelial cells that express a protein pattern corresponding to gene expression in response to VEGF and oestradiol only, which is totally different from those of cells disclosed in US patent MAYUMI et al. and/or HEBBEL et al.

Thus, it would never occur to a skilled person to use cells (derived from aorta) and to culture them in the presence only of VEGF and oestradiol to provide specific antibodies.

One of ordinary skill and creativity would thus fail to produce claim 36 of the present invention from a knowledge of MAYUMI et al. and HEBBEL et al. A *prima facie* case of unpatentability has thus not been made.

This rejection is believed to be overcome, and withdrawal thereof is respectfully requested.

Conclusion

The rejections are believed to be overcome, obviated or rendered moot, and no issues remain. The Examiner is accordingly respectfully requested to place the application in condition for allowance and to issue a Notice of Allowability.

Prior art of record but not utilized is believed to be non-pertinent to the instant claims.

The Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 25-0120 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17.

Respectfully submitted,

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